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EVALUATION OF STABILIZING EFFECT FOR SEVERAL MONOCLONAL ANTIBODY IMMOBILIZED QUARTZ CRYSTAL MICROBALANCE BY STABILIZER REAGENTS

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ABSTRACT

We tested five stabilizers for remaining immunologic activity of anti-dinitrophenol (DNP), anti-C-reactive protein (CRP), and anti-2,3,7,8 tetrachlorodibenzo-*p*-dioxin (TCDD) antibody immobilized QCM under several storage conditions. Investigated nine stabilizers were as following; 0.25 % BlockAce® and StabilGuard® as commercial available reagents, 0.2 % glycerin and 0.2 % Bovine Serum Albumin (BSA) as conventional stabilizers, 0.2 % MPC copolymer stabilizer as developed reagent, and PBS solution as blank reagent. According to the experimental results, we found MPC copolymer (NOF Corp., Japan) coated QCM showed highly immunologic activity through specific antigens and their antibodies after the heat acceleration test and long-term storage.

INTRODUCTION

Immunoassay is known as one of the most important analytical methods; it is widely used in environmental analysis and biochemical studies because of its extremely high selectivity and sensitivity [1-2]. Especially, the QCM immunosensor technique offers benefits because it does not require a labeled reagent for detection of molecular interaction [3-4]. QCM immunosensor has been used a protein as an antibody-stabilizing reagent (stabilizer). Bovine serum albumin (BSA), casein hydrolysis, and serum have been used to prevent non-specific protein adsorption and restrict conformational change of immobilized antibody on QCM. However, stabilizers from protein easily suffer denaturation by increasing temperature and long-term storage in air. Those factors induced antibody denaturation and reduced immunologic activities of immobilized antibody on QCM. To construct a novel QCM immunosensor, the antibody should be immobilized without (or with minimal) decreased immunologic activity by use of a stabilizer for antibody. As advanced stabilizers, polymers having phospholipids polar groups, 2-methacryloyloxyethyl phosphorylcholine (MPC) polymers, are well known for prevention of antibody denaturation and suppression of protein adsorption on the solid phase of ELISA method [5-6]. We were compared five stabilizers, BlockAce®, glycerin, MPC copolymer stabilizer, StabilGuard®, and BSA, to maintain immunologic activity of immobilized antibody on QCM. Among stabilizers, MPC copolymer stabilizer contain the water-soluble amphiphilic phospholipid polymer as poly [2-methacryloyloxyethyl phosphoryl choline (MPC)] derivatives, reported that maintain highly stabilizing effect of antibody immobilized on the solid phase substrate [7]. Three different species of monoclonal antibody: anti-DNP, anti-CRP, and anti-Dioxin antibody, were compared in terms of their stabilizing effect. Using several stabilizers and antibodies, the effect of stabilizers was investigated as immunologic

activity of antibody on QCM with experiments of temperature acceleration, long-term storage, and calibration curve.

MATERIALS AND METHOD

Five stabilizers were investigated to maintain immunologic activity of three kinds of immobilized antibodies on QCM. Stabilizers were provided from NOF Co., Japan: 0.25% BlockAce® in PBS; 0.2% MPC copolymer stabilizer; StabilGuard®; and BSA (bovine serum albumin). Figure 1 shows chemical structure of MPC copolymer. In this study, MPC copolymer stabilizer was synthesized between MPC and BMA monomer (m:n = 5:5). For chemical modification of the QCM surface, cysteamine hydrochloride, glutaraldehyde and glycine were purchased from Wako Pure Chemical Co., Ltd. (Osaka, Japan). As antigens, we purchased 2,4-dinitrophenol (DNP), 2,4-dinitrophenylated albumin from bovine serum (DNP-BSA) from Wako Pure Chemical Co., Ltd. (Osaka, Japan), recombinant human C-reactive protein (CRP) from Oriental Yeast Co., Ltd., (Osaka, Japan), and 2,3,7,8-tetrachlorodibenzo-*p*-dioxin conjugated ovalbumin (Dioxin-albumin) from Research Diagnostics, Inc. (USA), respectively. Anti-DNP monoclonal antibody (Japan Bio-Test Lab. Co., Japan), anti-human CRP monoclonal antibody (Oriental Yeast Co., Ltd., Japan), and anti-Dioxin monoclonal antibody (Research Diagnostics, Inc. USA) were used to compare immunologic activity according to different species of immobilized antibody on QCM with stabilizer. All chemicals were used without further purification. Ultrapure water (18 MΩ cm^{-1}) prepared with Milli-Q (Millipore Ltd., Tokyo, Japan) was used throughout all processes. To immobilized antibody on gold surface of QCM, experimental methods were well described at our previous paper [8].

AT-cut of QCM with gold electrodes (9 MHz, 8 × 8 × 0.15 mm^3) was obtained from Nihon Dempa Kogyo Co., Ltd. (Tokyo, Japan). A laboratory-made oscillation circuit was used [9]. A computer (NEC PC9821, Tokyo, Japan) was employed for controlling the universal counter (Iwatsu SC7201, Tokyo, Japan) and recording QCM oscillation frequency. An incubator (Yamato IN600, Japan) was used to control air temperature.

RESULTS AND DISCUSSION

Three kinds of immobilized monoclonal antibodies (anti-DNP, anti-CRP, and anti-dioxin) on QCM with five stabilizers were used to consider both immunologic activity under temperature acceleration and long-term stability. To evaluate respective efficiency of four stabilizers, firstly, the experiment was done under the condition of temperature acceleration because temperature was most effective in inducing structural change and immunologic activity loss of antibody. Also, BSA, as a representative of a conventional stabilizer containing protein, was applied on QCM under the same condition. After treatment with each stabilizer, antibody immunologic activities were measured as the frequency shift of antigen-antibody binding after 2 h acceleration at 55°C. Figure 2 shows results of temperature acceleration after 2 h at 55°C in air. In three different species of immobilized antibody, QCM indicated almost identical response to each stabilizer. The difference in chemical structure of monoclonal antibody comprises only amino acid residues on hinge regions; the difference of amino acid residues causes specific binding for different antigen molecules. These results imply that the stabilizer acts to maintain the structure of all monoclonal antibodies with equal efficiency. For blank, the low immunoreaction response was considered not to be a remaining immunologic activity. Responses of MPC polymer and StabilGuard® show about 60% immunologic activity after 2 h storage at 55°C in air, while the response of BSA was indicated as below 30% of immunologic activity. After temperature acceleration, BSA was almost denatured; therefore, it did not function as a stabilizer for immobilized antibody on QCM. This fact indicated that artificial stabilizers were more effective than conventional stabilizers made from protein for

temperature increase in air.

In this study, we investigated the stabilizing effect of stabilizers for immobilized antibody on a QCM immunosensor coated with stabilizer. Stability was investigated for several immobilized antibodies on QCM in test conditions of temperature acceleration. Experimental results indicated that MPC copolymer stabilizer successfully achieved a stabilized QCM immunosensor for temperature acceleration. A QCM immunosensor treated with this superior stabilizer would be a candidate to commercialize a sufficiently efficient analytical tool.

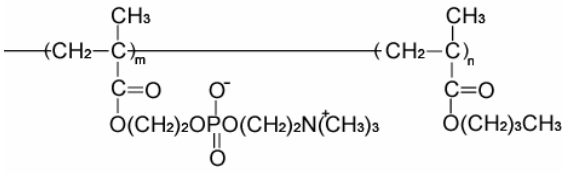

Fig. 1 Chemical structure of amphiphilic MPC copolymer.

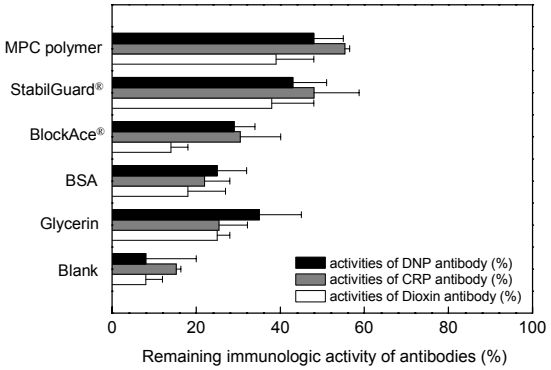

Fig. 2 Comparison of maintaining effect of stabilizer for three different species antibodies after 2 h storage at 55 ºC. DNP conjugated albumin, CRP, and 2,3,7,8-TCDD ovalbumin, with all the same concentration as 100 ng/mL, were applied into immobilized antibodies, respectively.

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